Production of essential oils from different species in two localities of Aragón, Spain

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Abstract

The demand of essential oils (EO) is increasing due to the interest of different industries (agrifood, cosmetic and pharmaceutical) in substituting chemically-synthesized constituents. Thus, it is necessary to cultivate medicinal and aromatic plants (MAPs) in a standardised way, in order to ensure the constant and homogeneous production for the industry. Moreover, the need to protect plant biodiversity, create an opportunity for farmers and foresters to diversify their production and improve their income. Five species (*Satureja montana* L., *Origanum vulgare* L. subsp. *virens*, *Salvia officinalis* L., and *Lavandula* × *intermedia* Emeric ex Loisel 'Grosso' and 'Super') were cultivated and harvested during four years in two localities, Bernues and Ejea de los Caballeros (Aragon, Spain). EO distillation was conducted at pilot plant scale, in a semi-industrial steam drag with at least 50 kg of dry biomass. The results of content and chemical characterization of the respective essential oils will be presented. Moreover, similar EO production was observed between locations for the rest of the species and no significant differences were observed in the average content of the different species between localities due to the fluctuations in the individual yields, which could be attributed to the climatic conditions of each season. This suggests that the species evaluated can be an alternative crop for different edaphoclimatic zones of Aragon with an essential oil content in the average of the selected species.

Keywords: crop yield, *Origanum*, GC-MS, steam distillation, alternative crops

INTRODUCTION

Plants have been found of great importance due to their medicinal and nutritional properties with a primary source of bioactive compounds. Many medicinal and aromatic plants (MAPs) contain a broad range of bioactive compounds (Lubbe and Verpoorte, 2011), depending not only on genetic factors, but also on factors such as environmental conditions and agricultural practices. In the case of aromatic crops, drought may cause significant changes in some metabolites yield and compositions (Petropoulos et al., 2008).

In a wide range of experiments, it could be shown that plants exposed to drought stress indeed accumulate higher concentrations of secondary metabolites (Selmar and Kleinwächter, 2013). However, it has to be considered that drought stress also reduces the growth of most plants. Due to the reduction in biomass, EO concentration enhances. Unfortunately, in most of the studies published, no data on the overall biomass of the plants analyzed are reported (Selmar and Kleinwächter, 2013).

Given the demand for a continuous and uniform supply of MAPs and the accelerating depletion of forest resources, domestication contributes to conserving its biodiversity. The application of sustainable methods of crop cultivation suitable for commercial large-scale production of safe and high-quality herbal material is of pivotal importance. Thus, sustainable and low input farming seems a good alternative.

The main species of the genus *Lavandula* from which commercial essential oil (EO) is obtained is called Lavandin (*L.* × *intermedia* Emerik ex Loisel) with an estimated average annual production of about 1000 t (Fernández-Sestelo and Carrillo, 2020). Currently a great

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part of utilized plant material from *Origanum vulgare* to produce essential oil are obtained directly from the environment, causing a negative effect on the wild populations, resulting in a lack of quality of the final products (Makri, 2002). *Satureja montana* L., commonly called winter savoury, is mainly cultivated and used on an industrial scale with antimicrobial, antifungal, antioxidant, antispasmodic, antiviral, and antidiarrheal potential (Caprioli et al., 2019; Jafari et al., 2016; Tepe and Cilkiz, 2016). *Salvia officinalis* has been highly valued for its aromatics and medicinal properties since ancient times and is one of the most important species for the production of essential oils, together with Salvia sclarea and *Salvia lavandulifolia* (Lubbe and Verpoorte, 2011). The whole plant is highly aromatic, the inflorescences in particular, and the essential oils possess a fresh, floral fragrance (Cai et al., 2006).

The aim of this work was to assess, through four-year field experiment (2017-2020), the effect of environmental differences and dripping irrigation at productive characteristics as well as on EO content (EOC) and quality for some Mediterranean aromatic species (*Satureja montana* L., *Origanum vulgare* L., *Salvia officinalis* L., and *Lavandula* × *intermedia* Emeric ex Loisel grosso (lavandin grosso) and super (lavandin super)), in two localities of Aragon, Spain.

MATERIALS AND METHODS

Plant material

Satureja montana L., *Origanum vulgare* L., *Salvia officinalis* L., and *Lavandula* × *intermedia* Emeric ex Loisel grosso and super plants were obtained in preliminary field trials in Aragon (Spain), from a pre-selected genetic material (Burillo, 2003). Two experimental assays located in Bernues (42°28’41.8”N 0°35’05.6”W / 917 m a.s.l) and Ejea de los caballeros (42°8’8.73”N, 1°12’31.50”W / 346 m a.s.l.) were established: 50 plants of each species, planted in line, with an intra-row spacing of 0.50 m. Each plant was considered an experimental unit. In Ejea de los Caballeros, due the low rainfall in the area, drip irrigation was provided from June to August (drippers every 30 cm, 4 L h^{-1}, 5-6 h $week^{-1}$). Bernues assay was grown under rainfall, characterized by an annual rainfall average of 697.8 mm.

During 4 years, plants were harvested yearly at 75% of blooming, manually, to evaluate their fresh biomass production, essential oil content (EOC) (%) (Equation 1) obtained in a pilot plant steam distillation at Agrifood Investigation and Technology Centre (CITA) and chemical composition.

$$EOY\ (\%) = (VEO/WP) \times 100 \qquad (1)$$

Where VEO is the volume obtained of EO (mL) and WP is the weight of distilled plant (g) in dry basis.

Essential oil content and chemical characterization

A stainless-steel pilot plant steam distillation was carried out to extract OIL from the fresh biomass (50 kg fresh plant biomass $batch^{-1}$) at a working pressure of 0.6 bar (Julio et al., 2017). Then EOC and composition from the different species and locations were assessed.

EOC was determined (Equation 1) after steam distillation and an aliquot was subjected to gas chromatography-mass spectrometry (GC-MS) to determine the EO composition. They were analyzed by GC-MS using a Shimadzu GC-2010 gas chromatograph coupled to a Shimadzu GCMS-QP2010 Ultra mass detector (electron ionisation, 70 eV) and equipped with a 30 m × 0.25 mm i.d. capillary column (0.25 µm film thickness) Teknokroma TRB-5 (95%) Dimetil-(5%) diphenylpolisiloxane. Working conditions were as follows: split ratio (20:1), injector temperature 300°C, temperature of the transfer line connected to the mass spectrometer 250°C, initial column temperature 70°C, then heated to 290°C at 6°C min^{-1}. Electron ionisation mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the Wiley 229 Mass Spectral Database. Retention times/indices of authentic compounds were also used to confirm the identities of the constituents. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

Statistical analysis

Data were analyzed and subjected to two-way ANOVA (GraphPad Prism) to evaluate the effect of plant species and location factors and a Tukey's multiple comparison test was used to determine whether there were significant differences ($p<0.05$) among means.

RESULTS AND DISCUSSION

Biomass results (Figure 1A) were strongly different between the two localities for all the species, ranging from 1.81±0.42 kg plant^{-1} in Ejea to 0.05±0.03 kg plant^{-1} in Bernues for lavandin grosso, and from 2.03±0.37 kg plant^{-1} in Ejea to 0.12±0.06 kg plant^{-1} in Bernues for lavandin super. Both lavandins presented the highest performance in Ejea, but the lowest in Bernues compared with the rest of species studied. Moreover, biomass from *S. montana* and *S. officinalis* were 0.92±0.23 kg plant^{-1} in Ejea and 0.14±0.06 kg plant^{-1} in Bernues, and 1.24±0.37 kg plant^{-1} in Ejea and 0.17±0.04 kg plant^{-1} in Bernues, respectively. Finally, the biomass obtained from *O. vulgare* subsp. *virens*, which did not present significant differences between both localities, ranged from 0.43±0.07 kg plant^{-1} in Ejea to 0.18±0.09 kg plant^{-1} in Bernues.

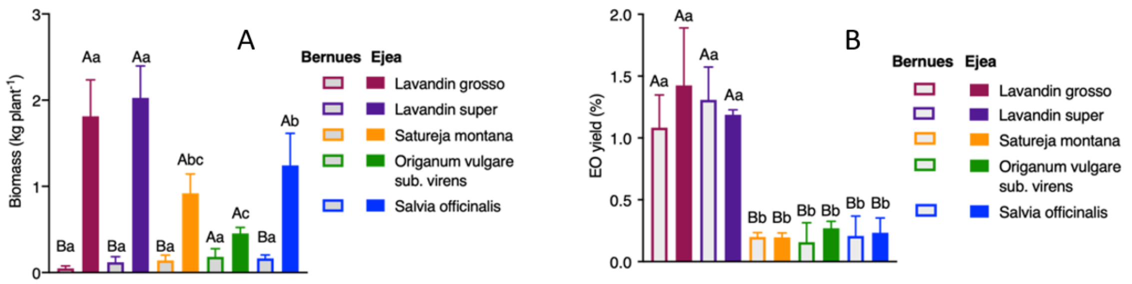

Figure 1. Biomass (kg plant^{-1}) (A) and essential oil (B) content obtained in Bernues and Ejea de los Caballeros for the different species studied during four years. Results are presented as the mean of four years ± standard deviation. Same capital letters indicate no difference ($p>0.05$) between localities for the same MAP species. Same lowercase letters indicate no difference ($p>0.05$) between species for the same locality.

The differences in biomass may be due to the total rainfall plus irrigation that makes possible a better annual distribution of water. Bernues doubled the rainfall of Ejea de los Caballeros the two first years (average of 750 mm in Bernues and 330 mm, respectively). Despite the highest rainfall of Bernues, the increase of Biomass seems to depend more on a better distribution of water availability. For that reason, a minimum dripping irrigation, used in Ejea de los Caballeros during summer, guarantees biomass production. Aziz et al. (2008) showed irrigation had a significant effect in plant growth of *Thymus vulgaris* plants, adding the fact plant growth depend on cell division, an event strongly affected by water availability (Kusaka et al., 2005).

The EOC of the studied species (Figure 1B) obtained in Ejea de los Caballeros ranged from 0.93% to 2.05% for lavandin grosso, from 1.16 to 1.25% for lavandin super, from 0.16 to 0.24% for *S. monatana*, from 0.22 to 0.33% for *O. vulgare* subsp. *virens*, and between 0.15 and 0.37% for *S. officinalis*. In general, at Bernues, EOC were slightly lower than in Ejea, ranging from 0.90 to 1.27% for lavandin grosso, from 1.15 to 1.61% for lavandin super, from 0.16 to 0.33 for *S. montana*, from 0.05 to 0.34% for *O. vulgare* subsp. *virens*, and between 0.10%) and 0.39% for *S. officinalis*. Other studies showed drip irrigation influenced EOC (Aziz et al., 2008; Salata et al., 2020).

No significant differences were observed in the average EOC for both localities due to the great variance observed in the amount of EO obtained depending on environment conditions. Since a number of factors have been known to affect the essential oil content including geographical location, developmental phase, solar radiation, harvesting time and growth conditions (Morshedloo et al., 2018), the differences in the EO composition among the different production areas may be due to the developmental stages or variations in cultivation conditions caused by specific environmental factors or drought stress, in the case of Bernues.

Table 1 presents the main compounds identified for the different plant species. *S. montana* main compounds for Bernues and Ejea de los Caballeros were carvacrol (40.18±4.05% and 37.40±0.57%), p-cymene (15.86±4.76% and 22.52±3.57%), γ-terpinene (10.99±6.53% and 8.60±2.23%) and thymol (4.88±1.66% and 8.44±1.37%). Their abundance (%) varied depending on cultivation site but also with the collection year, being the highest in Bernues for γ-terpinene and carvacrol, and in Ejea de los Caballeros for p-cymene and thymol. EO of *O. vulgare* subsp. *virens* cultivated in Bernues was characterized by its content in γ-terpinene (21.61±5.08%), which increased during the period studied. Other main components were linalool (8.31±5.10%) and β-caryophyllene (8.11±2.57%). However, the same species cultivated in Ejea de los Caballeros presented lower linalool content (0.81±0.49%), but significantly higher abundance of thymol (18.77±3.89%) than those of Bernues. Indeed, biotic and abiotic stress influences levels of secondary metabolites in plants (Dixon and Paiva, 1995).

S. officinalis EOs from Bernues were characterized by β-thujone (17.04±1.77%), β-caryophyllene (12.52±1.29%), α-humulene (12.89±1.43%) and viridiflorol (12.69±1.42%). On the other hand, EOs from Ejea de los Caballeros had β-thujone (12.77±1.07%), β-caryophyllene (9.01±0.96%) and 1,8-cineole (9.71±2.84%) as main compounds, presenting lower abundances of the former ones compared to the other location. Finally, lavandin EOs were characterized by linalool, linalyl acetate and camphor in both locations, but also by 1,8-cineole in lavandin grosso (7.10±1.10%) and super (7.22±1.36%) EO obtained from Ejea de los Caballeros. Lavandin grosso and super from Bernues had 28.92±1.56% and 25.53±4.36% of linalool, 45.36±11.50% and 45.22±4.52% of linalyl acetate, and 6.15±0.26% and 5.16±0.87% of camphor, respectively. In Ejea de los Caballeros, lavandin grosso and super EOs showed 27.17±2.28% and 29.26±2.09% of linalool, 31.62±3.67% and 36.61±2.85% of linalyl acetate, and 7.84±0.23% and 5.90±0.51% of camphor, respectively.

CONCLUSIONS

Overall, the average biomass (kg plant^{-1}) was much bigger in Ejea de los Caballeros for all species than in Bernues. Nevertheless, EOC did not follow the same tendency. Further studies must be done to prove the fact that more availability of water during summer did not improve EO content, indeed, some species increased their EOC in Bernues (lavandin grosso and super). This could be a positive result for farmers of drylands due to future possibilities of diversification. On the other hand, if the final production is dried plants (herbs, teas…), irrigation is mandatory in the environment conditions studied, especially for species that presented the lowest values for essential oil, *S. montana*, *S. officinalis* and *O. vulgare virens*.

Composition of EO was affected by the environment, changing the main compounds in different locations, for some species like *O. vulgare virens* and *S. officinalis.* Lavandin presented good concentrations for linalool and linalyl acetate, and *S. montana* for carvacrol (<50%), which is an advantage for the EO market.

Industrial cultivation of MAPs to obtain essential oil could create opportunities for development of agriculture in drylands where growing traditional crops is not a feasible option. Also, the activity can avoid overexploitation of wild populations, misidentification, genetic and phenotypic variability, extract variability and instability, toxic components and contaminants.

Table 1. Essential oil content and abundance of main compounds of the different species studied in the two localities during the four-year trial.

Species	Compound	Concentration (%)							
		Bernues				Ejea de los Caballeros			
		2017	2018	2019	2020	2017	2018	2019	2020
S. montana	Essential oil content (EOC)	0.16	0.23	0.20	0.33	0.21	0.16	0.19	0.24
	α-terpinene	1.90	2.17	1.93	0.80		1.89	2.26	1.94
	p-cymene	11.75	12.30	17.57	21.82		23.52	18.56	25.48
	γ-terpinene	12.36	16.01	14.14	1.46		7.50	11.17	7.13
	L-borneol	1.31	1.05	0.85	0.88		1.60	1.44	1.39
	Thymol	7.19	4.85	3.36	4.10		8.14	9.94	7.25
	Carvacrol	41.32	43.75	41.29	34.36		37.00	38.05	37.14
	β-caryophyllene	6.07	3.31	3.37	6.41		3.74	4.14	3.90
	β-bisabolene	3.51	2.78	2.63	5.39		2.37	2.15	3.36
O. vulgare subsp. virens	Essential oil content (EOC)	0.34	0.05	0.09	0.18	0.33	0.22	0.25	
	α-terpinene	2.28	1.69	2.67	3.16	3.98	4.22	1.98	
	p-cymene	3.30	3.69	-	4.18	6.99	6.61	4.00	
	γ-terpinene	15.40	20.16	23.50	27.36	18.99	28.50	16.41	
	Linalool	15.30	3.16	6.71	8.07	1.15	-	0.46	
	Thymol	4.64	4.61	6.11	6.10	14.68	22.42	19.22	
	Carvacrol	2.88	2.03	5.56	3.38	-	-	-	
	β-caryophyllene	4.31	9.95	9.17	9.00	9.90	6.12	3.15	
	Germacrene-D	2.43	7.97	5.81	6.98	4.97	3.57	3.40	
S. officinalis	Essential oil content (EOC)	0.13	0.10	0.39		0.37	0.33	0.15	0.18
	α-pinene	2.20	1.14	2.38		6.03	8.37	10.00	4.37
	Camphene	1.50	0.92	1.72		2.88	3.04	3.79	3.51
	β-pinene	8.60	2.81	6.01		6.75	3.91	10.04	11.99
	1,8-cineole	7.71	5.67	8.25		8.46	6.38	11.23	12.76
	β-thujone	15.04	17.68	18.41		12.67	11.33	13.22	13.85
	Thujone	3.03	2.81	3.30		4.69	7.30	6.22	4.18
	Camphor	1.53	1.95	2.76		6.12	6.13	5.68	4.66
	L-borneol	3.45	2.08	3.28		4.42	3.84	4.96	3.85
	β-caryophyllene	13.59	12.88	11.08		8.84	8.82	8.05	10.34
	α-humulene	12.61	14.44	11.62		7.56	4.79	4.70	5.55
	Viridiflorol	13.13	13.84	11.11		10.38	8.05	5.31	7.81
	Manool	6.56	12.29	9.09		4.53	3.02	0.92	1.84

Table 1. Continued.

Species	Compound	Concentration (%)							
		Bernues				Ejea de los Caballeros			
		2017	2018	2019	2020	2017	2018	2019	2020
Lavandin grosso	Essential oil content (EOC)			1.27	0.90	1.39	2.05	1.33	0.93
	Limonene + β-phellandrene			0.76	0.55	-	0.13	0.65	-
	1,8-cineole			3.89	3.88	7.09	8.48	5.78	7.06
	Linalool			27.82	30.02	27.28	28.70	28.78	23.90
	Camphor			6.33	5.96	7.76	8.16	7.82	7.63
	Borneol			0.24	3.80	2.89	3.67	3.86	2.22
	4-terpineol			0.15	2.44	-	4.35	3.73	1.91
	Linalyl acetate			53.49	37.22	31.56	27.91	30.39	36.63
	Lavandulyl acetate			0.73	3.64	4.17	3.65	3.67	3.90
Lavandin super	Essential oil content (EOC)		1.16	1.15	1.61	1.17	1.25	1.18	1.16
	1,8-cineole		6.46	2.07	3.73	6.39	8.77	5.82	7.91
	cis-β-ocimene		1.19	1.98	1.62	1.53	-	1.20	2.03
	trans-β-ocimene		1.22	1.08	2.58	1.78	2.10	1.78	1.81
	Linalool		24.90	21.51	30.17	31.30	30.04	29.33	26.38
	Camphor		5.99	4.26	5.24	5.66	5.67	5.61	6.67
	L-borneol		2.88	3.36	3.37	2.93	2.98	3.20	3.11
	α-terpineol		0.17	0.67	0.60	1.74	1.49	0.72	0.87
	Linalyl acetate		44.36	50.11	41.20	36.45	32.74	39.42	37.83
	Lavandulyl acetate		3.11	2.73	2.48	2.55	2.34	2.29	2.87
	β-caryophyllene		2.68	1.98	1.92	2.03	2.05	1.96	2.40

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